Eur J Cancer, Vol. 26, No. 4, pp. 442–449, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00
Pergamon Press plc

The Prognostic Value of Immunohistochemical Estrogen Receptor Analysis in Paraffin-embedded and Frozen Sections Versus That of Steroid-binding Assays

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Estrogen receptors (ER) were independently analyzed using dextran-coated charcoal assays (ER-DCC) and immunohistochemical assays in frozen (ER-ICA) and paraffin-embedded tissue (ER-PAR) from 130 human breast cancer specimens drawn from postmenopausal high-risk patients registered in the Danish Breast Cancer Cooperative Group. ER was best detected with the ER-DCC assay followed by the ER-ICA (relative sensitivity 87%) and the ER-PAR assays (relative sensitivity 71%). The semiquantified staining features of the immunohistochemical assays were statistically significantly correlated with each other and with ER-DCC. Analysis of disease-free interval (DFI) and overall survival (OS) showed that all assays allowed statistically significant discrimination between a high risk and a low risk group, although the sensitivity differences tended to be reflected as small differences in clinical discriminatory power. The patient groups were then stratified according to adjuvant treatment [radiotherapy (RT) versus radiotherapy and tamoxifen (RT + TAM)]. The survival advantage was tied primarily to the receptor status itself in the steroid-binding assays, but was linked to both the receptor status and the adjuvant treatment in the immunohistochemical assays. Thus, the relative risks in terms of DFI and OS were of the same relative magnitude in the RT and RT + TAM groups for ER-DCC assays using a cut-off level of 10 fmol/mg cytosol protein, while there were large differences in the relative risks between RT and RT + TAM groups for ER-ICA and ER-PAR assays. We conclude that an ER assay in fresh tissue should be given first priority, but if there is no fresh tissue, an ER assay in paraffin-embedded tissue offers a reasonably good alternative as a prognosticator and an equivalent alternative as a predictor of the response to endocrine treatment.
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INTRODUCTION

A LARGE number of studies has established the clinical value of steroid hormone receptor analyses in breast cancer biopsies. With steroid-binding techniques, estrogen receptors (ER) will help predict the clinical course of breast cancer [1–3], although others have failed to notice any such prognostic value in untreated patients [4, 5] and yet others have found an initial advantage to be lost with time [6]. Similarly, ER analyses are valuable predictors of the response to hormonal treatment in patients with advanced breast cancer [7, 8], and the probability of response has been found to be proportional with the ER concentration in the primary tumor [9, 10]. Determination of the ER content will also make it easier to predict who will respond to adjuvant tamoxifen (TAM), but the response is reportedly confined to patients with an ER content of more than 100 fmol/mg cytosol protein [11, 12]. However, contradictory results have been published for adjuvant treatment as some studies have found the beneficial effect of TAM to be independent of the ER status [13]. Thus, conclusions on the role of ER in the management of breast cancer differ although an apparently identical assay methodology (ER-DCC) has been used. Apart from the effect differences in the composition of study populations may have, the divergences may result from differences in assay sensitivities and interpretation.

A completely different assay technique, ER-ICA, has been developed using monoclonal ER antibodies for determination of ER in lightly fixed cryosections [14]. Compared to the DCC assay, this technique is rapid, requires a minimal amount of tissue and has the potential advantage of disclosing tumor heterogeneity. Studies have shown an excellent technical correlation between ER-DCC and ER-ICA results [14–16], and such good correlations were also found in our own previous study [17], although we found a small proportion of ER-DCC positive specimens to be ER-ICA negative. All of these tumors had a relatively low (< 100 fmol) ER content [17]. In contrast, only few studies have addressed the clinical relevance of ER-ICA determinations and those which have have primarily dealt with

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the prediction of response to endocrine therapy in advanced breast cancer and the results have been comparable to those of DCC assays although no direct comparison has been attempted [15, 18, 19]. In a previous study where the prognostic potential of ER-ICA and ER-DCC was compared directly [17], the data were comparable concerning the disease-free interval (DFI) and the overall survival (OS). The follow-up period was relatively short (median 22 months) [17].

We have recently developed a method (ER-PAR) using monoclonal ER antibodies for determination of ER in sections of paraffin-embedded tissue [20, 21]. This technique has shown a good qualitative correlation with conventional binding assays, but the sensitivity was slightly lower in tumors with ER contents < 100 fmol/mg cytosol protein [20–22]. Clinical data published on this technique have shown a good prediction of response to endocrine therapy [23], but its clinical value has not been directly compared with that of ER-DCC and ER-ICA.

The technical advantages of these new immunohistochemical assays notwithstanding, the *clinical* value of results obtained with the new technologies should be documented and preferably directly compared with those of conventional techniques in the same patients before a general shift in assay principles can be undertaken. The aim of the present study was to create such a documentation by updating the clinical experience of our previous study comparing ER-ICA and ER-DCC [17] and especially to add information on technical and clinical results in paraffin-embedded tissue.

MATERIALS AND METHODS

Tissue

A total of 130 biopsies from patients with breast cancer registered in the Danish Breast Cancer Cooperative Group were studied [24]. The biopsies were sent for routine steroid receptor analysis at the Laboratory of Tumor Endocrinology, the Fibiger Institute Copenhagen. A part of the biopsy was immediately fixed in 10% buffered formalin for 24 h and used for verification of malignant tissue. Sections from this tissue block were used for ER-PAR analysis. The other part of the tissue was used for ER-DCC analysis and for ER-ICA analysis on frozen sections. ER-DCC data were available in 127 of the 130 biopsies (multipoint DCC: 121, two-point DCC: 6). ER-ICA data were available in 118 (pos./neg. classification in 118, semiquantified ER in 113) and ER-PAR data were available in 116 biopsies (all with semiquantified ER). Comparison of assays was based on the largest common denominator of any two assays.

The 130 biopsies were all from postmenopausal high-risk patients with intially operable breast cancer. High risk was defined as a tumor >5 cm in diameter and/or positive lymph nodes and/or skin or fascial invasion. The primary treatment was total mastectomy and partial axillary dissection. Postmenopausal was defined as menostasia for more than 5 years. Eligibility criteria for protocolled treatment were absence of any evidence of advanced disease as estimated from general physical examination, routine blood tests, chest X-rays, and bone X-rays or scintigraphy. Further criteria were absence of any other previous malignant disease and informed consent. One hundred and eight patients were entered in protocol, 84 in DBCG-77 and 24 in DBCG-82. In the DBCG-77 protocols, all patients received adjuvant radiotherapy (RT) to the chest wall, the axillary and periclavicular areas equivalent to 1335 rets and were then randomized to either observation or adjuvant tamoxifen 10 mg t.i.d. for 48 weeks (RT alone or RT + TAM). In the DBCG-82 protocols, patients were randomized to either (1) RT and TAM (same schedule as in the DCGB-77 protocols), (2) TAM, or (3) TAM + cytoxan, methotrexate, and 5-fluorouracil. In five of the 108 protocolled patients, receptor analyses were made in a metastasis rather than in the primary tumor (all five analyses from patients in the DBCG-77 protocols). Material from the remaining 103 patients was used for clinical analysis of the prognostic value of the individual assays. With deduction of failed analyses, clinical data and receptor status of the primary tumor were available in 101 patients with ER-DCC, 94 patients with ER-ICA, and 93 patients with ER-PAR. Finally, stratification for adjuvant treatment showed that 40 of the 103 patients had received RT alone and 44 RT + TAM. Of these 84 patients, the receptor status of the primary tumor was determined in 82 patients with ER-DCC, 78 patients with ER-ICA, and 76 patients with ER-PAR.

Follow up was conducted as previously described [24]. The patients entered the study between September 1981 and June 1983. The clinical data were evaluated as of 1 December, 1988 at which time the median observation period was 75 months. The disease-free interval (DFI) and the overall survival (OS) was calculated as the period from mastectomy until the date of recurrence (DFI) or death (OS).

Steroid receptor analysis

DCC. The estrogen and progesterone receptors were measured by a DCC assay as recommended by the EORTC [25] with the modifications previously described [26]. Tumors were considered receptor positive if at least 10 fmol/mg cytosol protein were present. In all cases the receptor content was measured in histologically verified malignant tissue. All assay results were evaluated by the same co-author (S.M. Thorpe).

ER-ICA. Immunocytochemical analysis of frozen tissue was performed as previously described in detail [14, 17]. Clasification of receptor status and evaluation of semiquantitative staining features (staining intensity and percent ER positive cells) have previously been described [14, 17]. In brief, the intensity of the nuclear staining was subjectively scored 0, 1, 2, 3, with 1 representing faint but distinct staining, recognizable above controls, 3 representing the most intense staining seen, and 2 an intermediate. The perecentage of ER positive epithelial cells was estimated with a microscopic grid by counting the number of grid points falling over positive nuclei relative to the points falling over unstained nuclei. At least 200 cells were

Table 1. Comparison of receptor status in paraffin sections (ER-PAR), frozen sections (ER-ICA), and DCC assay (ER-DCC)

Assay	Estrogen receptor	
	Positive	Negative
ER-PAR	68 (59%)	48 (41%)
ER-ICA	85 (72%)	33 (28%)
ER-DCC	98 (77%)	29 (23%)

Table 2. Qualitative relationship between immunohistochemical and biochemical ER assays in human breast tumors

ER-DCC	ER-PAR Positive	ER-PAR Negative
Positive	65	26
Negative	3	21

$P < 10E\text{-}6$ (Fisher's exact test). Overall agreement 75%.

ER-DCC	ER-ICA Positive	ER-ICA Negative
Positive	80	12
Negative	3	21

$P < 10E\text{-}6$ (Fisher's exact test). Overall agreement 87%).

ER-ICA	ER-PAR Positive	ER-PAR Negative
Positive	62	17
Negative	3	27

$P < 10E\text{-}6$ (Fisher's exact test). Overall agreement 82%.

Table 3. Comparison of ER-DCC concentrations and potential biological differences [ER-DCC equilibrium dissociation constant (K_d), and PgR-DCC] in tumors with discordant ER status in immunohistochemical and steroid binding ER assays. Only ER-DCC positive tumors with ER concentrations ≤ 100 fmol analyzed

	ER-PAR Positive	ER-PAR Negative	ER-ICA Positive	ER-ICA Negative
ER-DCC				
Average ranks*	23.6	19.5	20.8	13.8
No. of tumors†	15	26	24	12
Probability‡	0.30		0.06	
ER-DCC K_d				
Average ranks*	16.7	22.7	16.3	18.8
No. of tumors†	15	25	24	9
Probability‡	0.11		0.52	
Pgr-DCC				
Average ranks*	19.0	20.5	19.8	12.5
No. of tumors†	14	25	23	11
Probability‡	0.71		0.05	

*Mann–Whitney rank sum test of receptor concentrations.
†All data not available in all tumors.
‡Two tailed probability.

counted from each tumor. All assay results were evaluated by the same co-author (W.J. King).

ER-PAR. Immunocytochemical analysis of paraffin-embedded tissue was performed as previously described in detail [20, 21]. Classification of receptor status and evaluation of semiquantitative staining features have previously been described [20, 21]. We used the same technique for semiquantitation of the staining intensity and the same way of calculating the percentage of positive cells in the ER-ICA and the ER-PAR assays. All assay results evaluated by the same co-author (J. Andersen).

The specificity of the monoclonal ER antibodies used in ER-ICA and ER-PAR has previously been documented [27–29].

Table 4. Correlations between semiquantified immunohistochemical staining features (scoring: See Materials and Methods) of paraffin-embedded tissue (ER-PAR), frozen tissue (ER-ICA) and the ER content (ER-DCC) of 130 breast tumors. Correlation coefficients were obtained by simple linear regression analysis. Log (ER-DCC + 1) was used in correlations

	ER-PAR Staining intensity	ER-PAR Percentage positive cells	ER-ICA Staining intensity	ER-ICA Percentage positive cells	ER-DCC
ER-PAR					
Staining intensity	*	0.83	0.69	0.64	0.74
Percentage positive cells		*	0.66	0.61	0.62
ER-ICA					
Staining intensity			*	0.85	0.76
Percentage positive cells				*	0.81

All correlation coefficients significantly different from 0 at 0.01 probability level. No significant differences between correlation coefficients at 0.05 probability level when compared pairwise.

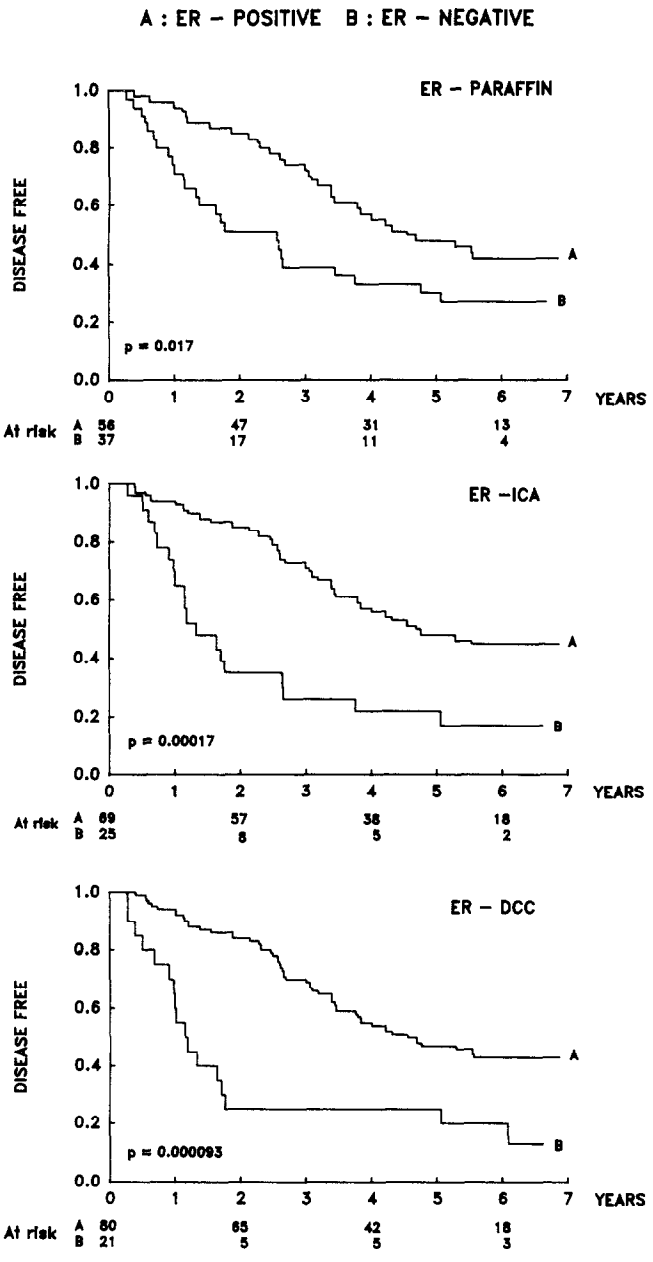

Fig. 1. Disease free interval and ER-PAR status (top), ER-ICA status (middle), and ER-DCC status (bottom).

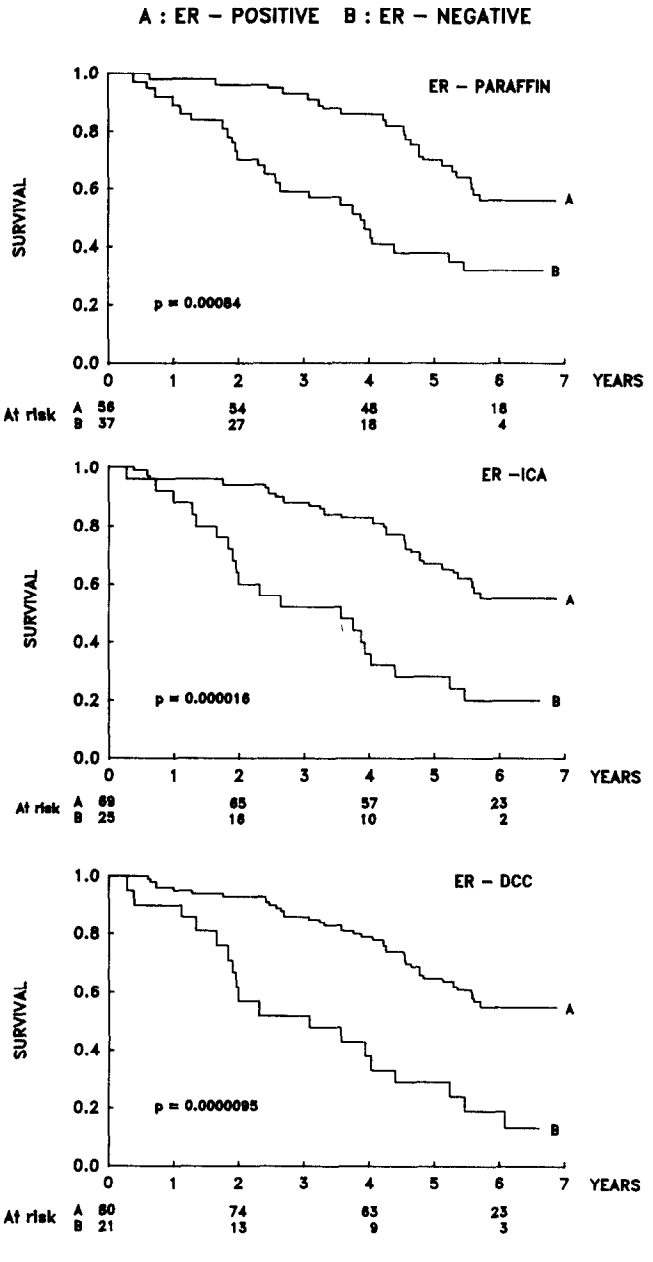

Fig. 2. Overall survival and ER-PAR status (top), ER-ICA status (middle), and ER-DCC status (bottom).

Statistics

Linear regression was used to correlate the immunohistochemical staining features with cytosolic ER content determined with the DC assay. The correlation coefficient, r, was obtained from a least squares fit. Comparison of two correlation coefficients was performed as suggested by Hald [30]. Estimation of DFI and OS was performed by life table analysis and the significance of differences between selected groups was assessed using log-rank analysis [31]. Estimation of the relative risk in selected patient groups was performed as suggested by Crowley [32]. Analysis of contingency tables was performed using Fisher's exact test. The Mann–Whitney rank sum test was used to test for different centerings of the underlying distribution of biochemical receptor data in groups defined by immunohistochemical staining.

RESULTS

Comparison of assays

Table 1 shows the ER-DCC assay to detect ER most frequently followed by ER-ICA and ER-PAR. There was a highly statistically significant association between the ER classification of all three assays, with the best overall agreement obtained

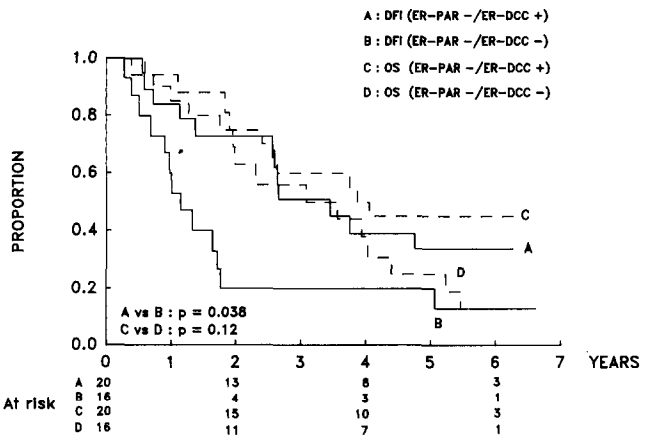

Fig. 3. Disease free interval and overall survival in patients with ER-PAR negative status and concordant or discordant ER-DCC status.

between ER-DCC and ER-ICA followed by ER-PAR and ER-ICA and ER-PAR and ER-DCC (Table 2). The disparities were mainly due to tumors classified as ER positive by the DCC assay and ER negative by the immunohistochemical assays. Thus, using the ability of the assays to detect ER as an (although indirect) measure of their sensitivity, ER-DCC was the most sensitive assay followed by ER-ICA and ER-PAR. Accepting the ER-DCC assay as being 'truth' for comparisons, the sensitivity of the ER-PAR assay was 71.4% [65/(65 + 26) × 100] and that of the ER-ICA assay 86.9% [80/(80 + 12) × 100].

The ER-DCC positive, ER-PAR negative tumors had a receptor content below 100 fmol/mg protein with one exception containing 135 fmol. The median ER content of these tumours was 23 fmol. The ER-DCC positive, ER-ICA negative tumors all had an ER content below 100 fmol (median 20 fmol). In the ER-DCC positive tumors with a receptor concentration below 100 fmol, where the ER-DCC assay and the immunohistochemical assays come up with conflicting ER status, further analysis of the tumors was performed separately after stratification according to the immunohistochemical ER status of the individual tumors. As shown in Table 3, there was no difference between the ER content of groups with a concordant or discordant ER-PAR status. Among the groups stratified by the ER-ICA assay, the average ranks of ER contents were considerably higher in the group having a concordant ER status, but the difference barely reached statistical significance. The equilibrium dissociation constant K_d was equally distributed in the groups stratified by ER-PAR or ER-DCC, but the average ranks of PgR concentrations were significantly higher in the ER-DCC positive/ER-ICA positive group than in the ER-DCC positive/ER-ICA negative group.

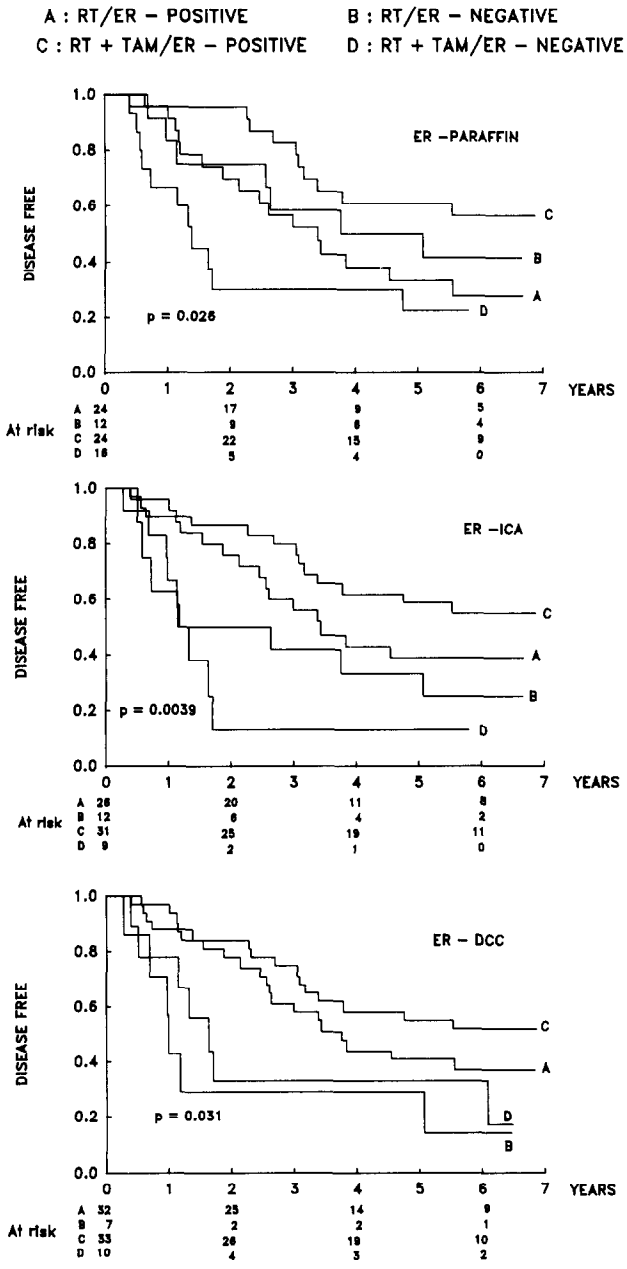

Fig. 4. Disease free interval, adjuvant treatment, and ER-PAR status (top), ER-ICA status (middle), and ER-DCC status (bottom). RT: radiotherapy; RT + TAM: radiotherapy and tamoxifen.

Table 5. The estimated relative risk of patients with receptor negative breast tumors compared with patients with receptor positive tumors. Comparison of receptor status obtained from immunohistochemical ER staining in paraffin-embedded tissue (ER-PAR) and frozen tissue (ER-ICA) with that obtained from biochemical ER-DCC assays

Parameter	Assay	Patients pos./neg.	Relative risk	Confidence limits*
DFI	ER-PAR	56/37	1.94	1.01–3.18
DFI	ER-ICA	69/25	2.90	1.41–4.93
DFI	ER-DCC	80/21	3.12	1.49–5.34
OS	ER-PAR	56/37	2.69	1.29–4.59
OS	ER-ICA	69/25	3.44	1.64–5.89
OS	ER-DCC	80/21	3.54	1.71–6.02

DFI: disease free interval; OS: overall survival.
*95% confidence limits.

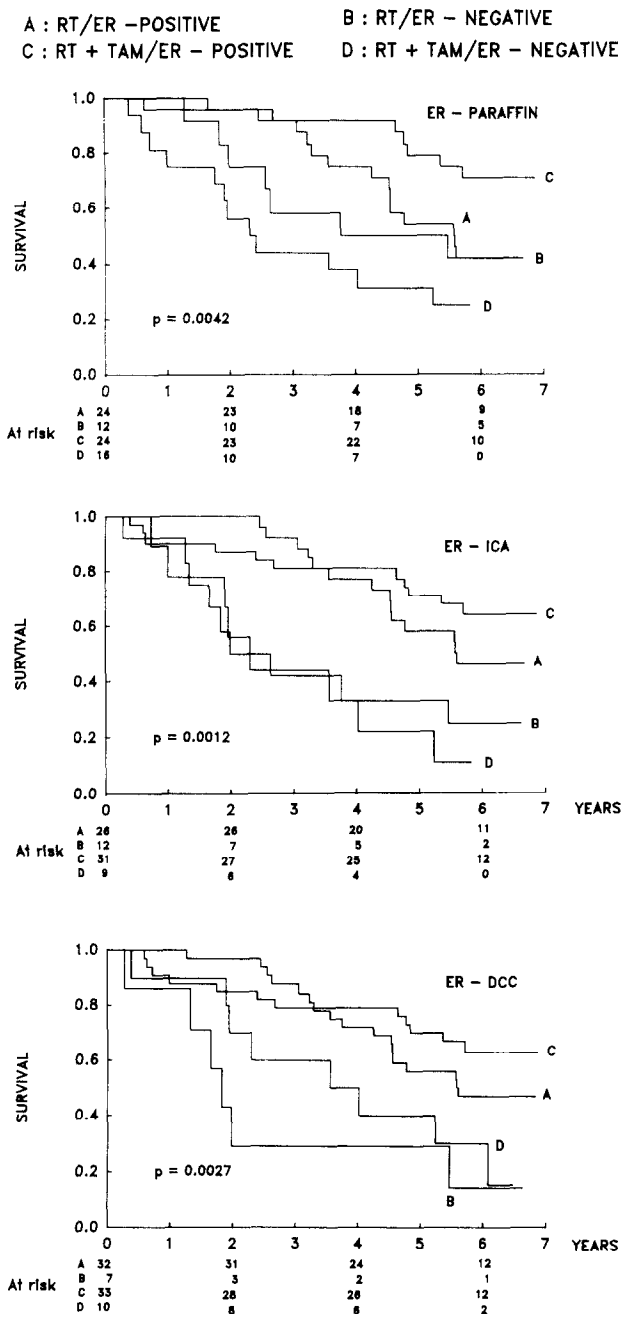

Fig. 5. Overall survival, adjuvant treatment, and ER-PAR status (top), ER-ICA status (middle), and ER-DCC status (bottom). RT: radiotherapy; RT + TAM: radiotherapy and tamoxifen.

The semiquantifiable staining features of the immunohistochemical assays were correlated with each other and with the DCC assay. The ER-DCC values were found to fit best to a log-normal distribution and log (ER-DCC + 1) was used as the dependent variable in the linear correlations. The correlation coefficients between assay variables are summarized in Table 4, where the staining features (percentage stained cells and staining intensity) of the immunohistochemical assays are shown to be significantly correlated with each other and with ER-DCC. Although the best correlations with the ER-DCC assay was obtained using the ER-ICA assay, there were no statistically significant differences between any of the correlation coefficients.

Comparison of assays and clinical parameters

Figure 1 shows the relationship between the disease-free interval and receptor status determined by the ER-PAR, the ER-ICA, or the ER-DCC assay. All assays could statistically significantly discriminate between a high-risk and a low-risk group, with the apparent trend that the above-mentioned sensitivity differences were reflected as differences in clinical discriminatory power (lower probabilities derived from the log-rank tests). The same picture was seen regarding the overall survival as shown in Fig. 2. The important difference between the assays was their ability to select the high-risk group. As can be seen from Figs 1 and 2, the survival curves for ER positive are more identical than those of ER negative patients. To quantitate these differences between the assays, an estimation of the relative risk θ was made within the individual assays. The results of this analysis, which is shown in Table 5, confirms the trend that can be read from the survival curves, but as can be seen from the confidence limits, there were no statistically significant differences between the assays. The clinical importance of the sensitivity difference between the ER-PAR and the ER-DCC assay was explored by comparing disease-free interval and survival for ER-PAR negative patients with concordant and discordant ER-DCC status. This is shown in Fig. 3, which confirms that the above-mentioned technical sensitivity difference is of some clinical importance.

The patient groups were then further stratified according to adjuvant treatment (RT alone versus RT + TAM). The estimated curves for DFI and OS are shown in Figs 4 and 5, and the corresponding estimates θ of the relative risks are given in Table 6. These data show that the survival advantage was tied primarily to the receptor status itself in the steroid-binding assays, but was linked to both the receptor status and the adjuvant treatment in the immunohistochemical assays. Thus, the relative risks in terms of DFI and OS were of the same relative magnitude in the RT and RT + TAM groups for the ER-DCC assay, while there were large differences in the relative risks between RT and RT + TAM groups for ER-ICA and ER-PAR assays. However, the small number of patients increases the risk of over-interpreting the results as suggested from the large confidence limits of θ. If a cut-off level of 100 fmol was chosen for the ER-DCC assay in the RT + TAM group, 19 patients were ER positive, 24 were ER negative and the relative risk of death increased to 6.72 (0.779–18.509).

DISCUSSION

A comparison of the receptor classifications showed the ER-DCC assay to be slightly more sensitive than the ER-ICA assay and somewhat more sensitive than the ER-PAR assay. The overall agreement between the ER-DCC and the ER-ICA assays was comparable to that generally reported in the literature as reviewed by Andersen *et al.* [21]. There was less agreement between the ER-DCC and ER-PAR assays in this study than in previous studies [20–22] due to the larger proportion of ER-DCC positive but ER-PAR negative tumors. As in our previous studies, these tumors contained <100 fmol ER/mg cytosol protein. The reason for the apparently lower sensitivity of the ER-PAR assay in this study is not entirely clear, but there

Table 6. The estimated relative risk of patients with receptor negative breast tumors compared with patients with receptor positive tumors. Comparison of receptor status obtained from immunohistochemical ER staining in paraffin-embedded tissue (ER-PAR) and frozen tissue (ER-ICA) with that obtained from biochemical ER-DCC assays. Relationship to adjuvant treatment.

		Radiotherapy			Radiotherapy and tamoxifen		
Parameter	Assay	Patients pos./neg.	Relative risk	Confidence limits*	Patients pos./neg.	Relative risk	Confidence limits*
DFI	ER-PAR	24/12	0.74	0.21–1.58	24/16	4.11	0.97–9.44
	ER-ICA	26/12	1.64	0.52–3.41	31/9	5.38	1.12–12.82
	ER-DCC	32/7	2.79	0.69–6.31	33/10	2.66	0.71–5.86
OS	ER-PAR	24/12	1.26	0.35–2.75	24/16	6.07	1.11–14.99
	ER-ICA	26/12	2.66	0.76–5.72	31/9	4.49	1.15–10.06
	ER-DCC	32/7	3.99	0.89–9.32	33/10	2.99	0.85–6.43

DFI: disease free interval; OS: overall survival. *95% confidence limits.

may be several explanations. First, the percentage of patients classified ER positive by the DCC assay was considerably higher in the present study (≈77%) than in our previous studies (≈66%) maybe because previous materials were drawn from both pre- and postmenopausal women but the present material only from postmenopausal women who have a higher overall rate of receptor positivity. Second, there may be differences in the interpretation of binding assays among the individual laboratories [33, 34]. Third, the lower sensitivity may be due to differences in tissue handling between the laboratory processing the present samples (The Laboratory of Tumor Endocrinology of the Fibiger Institute) and The Receptor Laboratory in Aarhus, which handled our previous materials. We have previously shown that differences in fixatives and fixation times influence the preservation of immunoreactive ER [21].

The immunohistochemical assays measure a fundamentally different property of the receptor than the DCC assay (the presence of a specific protein structure rather than the ability to bind a specific hormone), and the lower sensitivity of the immunohistochemical assays may reflect the presence of different receptor subtypes with different antigenic sites and possibly different biological properties. However, the K_d of the estrogen receptors did not differ among the groups of ER-DCC positive tumors with a concordant or discordant immunohistochemical ER status. Analysis of PgR (a marker of biologically active ER) did not reveal any differences between the subgroups stratified by the ER-PAR assay, but the PgR content was higher in the ER-ICA positive/ER-DCC positive group than in the ER-ICA negative/ER-DCC positive group. This is not so much an expression of the different biological properties of the receptors identified in the two groups as it is the likely result of an overall (and almost statistically significant) higher ER level in the group with concordant ER-DCC/ER-ICA status as the PgR content has been shown to increase with increasing ER concentrations [35]. Thus, our data support the idea that the difference between the results of the DCC and the immunohistochemical assays springs rather from different assay sensitivities than from different biological properties of the receptors identified.

Analysis of semiquantified ER variables was given a relatively low priority in this study whose main goal was the comparison of the clinical data. The ER-PAR assay was scored according to the same criteria as the ER-ICA assay, but the sections analyzed by the two assays were randomly localized within the tumor and thus subject to the inherent variations throughout the tumor [36]. An optimal comparison should involve analysis of neighboring sections and the use of a computerized image analysis system. In spite of these limitations, there were significantly correlated individual staining features in the two immunohistochemical assays. The magnitude of the correlation coefficients between the ER-DCC and the ER-ICA assays was equivalent to that reported in other studies, but somewhat lower between the ER-DCC and the ER-PAR assays than previously reported [21]. This was mainly due to the relatively large number of ER-PAR negative/ER-DCC positive tumors in this study.

All assays provided clinically useful information on DFI and OS. The ER-DCC and the ER-ICA assays yielded almost identical clinical information (Figs 1 and 2), but the ER-PAR assay came up with a smaller difference in DFI and OS between the ER positive and ER negative groups. The DFI and OS curves for ER positive patients were almost similar in all three assays, but the survival curve for ER negative patients in the ER-PAR assay flattens out at 0.3 compared with 0.15 and 0.2 in the other assays because it contains a number of ('good risk') patients classified ER positive by the other assays. This is confirmed by the subsequent analysis of the ER-PAR negative patient group shown in Fig. 3. This difference between the assays is in accordance with previous studies suggesting that for the prognosis, the relevant cut-off level for definition of receptor positivity is in the vicinity of 5 fmol ER/mg cytosol protein [3, 37]. As shown in other studies, some of the survival advantage of ER positive patients is apparently lost with time [5, 38].

Stratification of the patients according to adjuvant treatment shows that the paraffin assay only has a lower prognostic sensitivity in patients who have not received adjuvant hormonal treatment, whereas its power to predict response to endocrine therapy is equivalent to, or for the OS, even greater than that of the other assays. This finding matches the general assumption of a cut-off level for prognostication around 5 fmol in untreated patients [3, 37] (an ER level where the ER-PAR assay has sensitivity problems) contrasted with a cut-off level for prediction of response to therapy around 100 fmol [11, 12], where the classification of ER status does not differ among the different assays. As shown in Figs 4 and 5, ER positive patients stratified by any of the ER assays benefited from adjuvant TAM treatment

(survival curve C > A), and that this benefit was linked with the ER status as only ER positive patients benefited from the treatment (survival curve C > D). The effect was present both when considering DFI and OS. These findings correspond with those previously published from the DBCG protocols [11]. The predictive value of the DCC assay may be improved by choosing another cut off level than 10 fmol/mg cytosol protein for discrimination between receptor positive and receptor negative biopsies, as this quantity originally was somewhat arbitrarily chosen and reflected the sensitivity of the assay at that time.

In conclusion: The ER-DCC assay was slightly more sensitive than the ER-ICA assay and somewhat more sensitive than the ER-PAR assay. The clinical correlations showed the ER-DCC and ER-ICA to be equivalent prognosticators of DFI and OS followed by ER-PAR, and all three assays were equivalent in predicting response to adjuvant endocrine therapy. Thus, an ER-assay in fresh tissue should be given first priority, but if fresh tissue is unavailable, an ER assay in paraffin-embedded tissue offers a reasonably good alternative as a prognosticator and an equivalent alternative as a predictor of the response to endocrine treatment. This makes retrospective analyses of large patient populations treated with adjuvant therapy feasible.

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Acknowledgements—The authors wish to thank Abbott Laboratories for supplying monoclonal antibodies and Ms B. Hermansen for skillful assistance.